polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template, in a solution containing between about 10% and about 20% (v/v) glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C, and an annealing temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers.

C1 CONT.

2. (Amended) The method of claim 1, wherein the glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol is present in about 15% (v/v).

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8. (Amended) The method of claim 1, wherein oligonucleotide primers of various lengths are extended with specific nucleotide terminations in the presence of ddNTPs or their analogs for cycle sequencing.

C2

SUB D1

9. (Amended) A method for extending an oligonucleotide primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature below about 80°C, comprising the steps of:

(i) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template, a suitable

concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, and

(ii) effecting cycle primer extension reaction(s) at a temperature below about 80°C for a sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs.

Please add new claims 18-35 as follows.

--18. (New) The method according to claim 1, wherein the DNA polymerase has at least 95% homology to the amino acid sequence of SEQ ID NO:2.

19. (New) The method according to claim 1, wherein the DNA polymerase is expressed from a DNA sequence that has at least 95% homology to the DNA sequence of SEQ ID NO:1.

20. (New) The method according to claim 1, wherein the DNA polymerase has the amino acid sequence of SEQ ID NO:2.

21. (New) The method according to claim 1, wherein the DNA polymerase is expressed from a DNA sequence having the DNA sequence of SEQ ID NO:1.

22. (New) A method for extending an oligonucleotide primer or a pair of oligonucleotide primers using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature of about 70°C, comprising the step of mixing a template DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase during dye-labeled

terminator automated DNA cycle sequencing reduces the innate selective discrimination against the incorporation of fluorescent dye-labeled ddCTP and fluorescent dye-labeled ddATP, in a solution containing between about 10% and about 20% (v/v) glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C and an annealing temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers.

C3 CON't.

23. (New) The method of claim 22, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

24. (New) The method according to claim 22, wherein the DNA polymerase has at least 95% homology to the amino acid sequence of SEQ ID NO:4.

25. (New) The method according to claim 22, wherein the DNA polymerase is expressed from a DNA sequence that has at least 95% homology to the DNA sequence of SEQ ID NO:3.

26. (New) The method according to claim 22, wherein the DNA polymerase has the amino acid sequence of SEQ ID NO:4.

27. (New) The method according to claim 22, wherein the DNA polymerase is expressed from a DNA sequence having the DNA sequence of SEQ ID NO:3.

28. (Amended) The method of claim 22, wherein the glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol is present in about 15% (v/v).

29. (Amended) The method of claim 22, wherein oligonucleotide primers of various lengths are extended with specific nucleotide terminations in the presence of ddNTPs or their analogs for cycle sequencing.

30. (Amended) A method for extending an oligonucleotide primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature below about 80°C, comprising the steps of:

(i) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase during dye-labeled terminator automated DNA cycle sequencing reduces the innate selective discrimination against the incorporation of fluorescent dye-labeled ddCTP and fluorescent dye-labeled ddATP, a suitable concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, and

(ii) affecting cycle primer extension reaction(s) at a temperature below 80°C for a sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs.

31. (New) The method of claim 30, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

32. (New) The method according to claim 30, wherein the DNA polymerase has at least 95% homology to the amino acid sequence of SEQ ID NO:4.

33. (New) The method according to claim 30, wherein the DNA polymerase is expressed from a DNA sequence that has at least 95% homology to the DNA sequence of SEQ ID NO:3.

C3 CONT.

34. (New) The method according to claim 30, wherein the DNA polymerase has the amino acid sequence of SEQ ID NO:4.

35. (New) The method according to claim 30, wherein the DNA polymerase is expressed from a DNA sequence having the DNA sequence of SEQ ID NO:3.--

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REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

Claims 1, 2, 8 and 9 about are amended to address the Examiner's objections under 35 U.S.C. §112, second paragraph. Non-elected claims 12-17 are canceled without prejudice or disclaimer. New claims 18-35 are added to cover additional embodiments. Support for these amendments and new claims can be found in the specification at page 2, lines 9-12; page 4, line 14 to page 5, line 30; page 6, lines 1-5; page 11, line 16 to page 12, line 3; page 15, line 30 to page 16, line 7; page 24, lines 9-11; and original claim 3. No new matter is introduced, and entry and full consideration is requested. Upon entry of this amendment, claims 1-11 and 18-35 will be pending.

In the Office Action dated July 24, 2002, claims 1-11 were rejected under 35 U.S.C. §112, second paragraph, as indefinite. The Examiner raised a few questions regarding what is meant by claims 1, 2, 8 and 9. Regarding claims 1, 2, and 9, the Examiner inquired what is meant by "mixtures thereof"; in claims 8 and 9, the Examiner